Use of a Laser-equipped Centrifugal Analyzer for Kinetic Measurement of Serum IgG

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Use of a visible laser in an Aminco "Rotochem" centrifugal analyzer enables light-scattering measurements to be made with this instrument. The modification is technically simple, inexpensive, and requires no fundamental changes in basic instrument design. The laser-modified analyzer has been applied to the measurement of IgG in serum. A two-point kinetic analysis is used to quantitate IgG from a standard curve: protein concentration vs. change in intensity of the scattered light during a fixed time interval. Fourteen 20-μl samples can be simultaneously determined in 1.9 min. No sample blank corrections are required. Within-run precision studies, based in each case on 56 replicate measurements, yielded coefficients of variation of 3.8% and 4.8% for normal and abnormal pools, respectively.

Additional Keyphrases: AutoAnalyzer • continuous-flow method compared • "Rotochem" centrifugal analyzer • measurement of nanogram amounts of proteins

Imunochemical nephelometric measurement of specific proteins has been the subject of numerous investigations. Although several manual (I-6) and automated nephelometric (7-13) techniques have been reported previously, these methods invariably have involved equilibrium light-scattering measurements. Recently, the IgG-anti-IgG reaction has been studied as a model system in reference to the possible kinetic measurement of specific proteins (14). Development of new instrumentation, such as the centrifugal analyzer, has made automated kinetic measurements possible that are precise and accurate (15). The development and use of centrifugal analyzer systems for fluorometric and light-scattering measurements have been limited, until recently, to prototype models developed at the Oak Ridge National Laboratory (16).

We describe here the modification of an Aminco "Rotochem II" to enable automated near-forward¹ light-scattering measurements to be done, and the application of the laser-modified analyzer to the development of a kinetic nephelometric method for the quantitation of immunoglobulin G (IgG) in serum. This modification of the instrument is technically simple, inexpensive, and requires no major changes in basic instrument design. The kinetic quantitation of serum IgG in this way is precise, rapid, and inexpensive, and results correlate well with those obtained by a continuous-flow nephelometric method.

Materials and Methods

Apparatus. The instrument, a modified Aminco Rotochem II centrifugal analyzer (American Instrument Co., Silver Spring, Md. 20920), and its modification have been described in detail (17). The modification involves three basic changes: the tungsten-halogen lamp is replaced with a helium–neon gas laser (alignment of the laser is most easily accomplished with the rotor removed from the instrument), both lenses located in the optical path are removed, and a dark field is placed between the cuvettes and the detector.

Optical system. A relatively inexpensive helium–neon gas laser (Spectra Physics, Mountain View, Calif. 94040; Model 155, 632.8 nm) was used as a source in place of the tungsten–halogen lamp that is the standard source for the instrument. Although, in theory, a conventional incandescent source could be used, focusing problems and incident light of a relatively low intensity were expected. We therefore decided to use the highly collimated and very intense laser beam in order to eliminate these difficulties and to provide maximum sensitivity. The laser beam is directed through the cuvettes to the center of the dark field by means of reflectance from a mirror positioned at an angle of 45° to the incident beam.

¹ "Near-forward" refers to the fact that light scattering is being measured at small (but not zero) angles.

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dark field interposed between the cuvettes and the detector blocks out light coming directly from the source and thus only scattered light reaches the detector.

**Computer.** Data accumulation and averaging were controlled by a PDP/8M minicomputer (Digital Equipment Corp., Maynard, Mass. 01754).

**Reagents**

*Polyethylene glycol solutions* were prepared by dissolving 40 g of polymer in 1 liter of phosphate-buffered (10 mmol/liter, pH 7.4) physiological saline (9 g of NaCl per liter). The polyethylene glycol solution as described was used as a diluent throughout this investigation.

*Goat antihuman IgG* was purchased from Technicon Instruments Corp., Tarrytown, N. Y. 10591. Antigenic specificity was determined by immunoelectrophoresis against whole human serum.

*Pooled human serum* was assayed for IgG by a commercial laboratory (Technicon) by use of the International Reference Preparation for Human Serum Immunoglobulins as prepared by the World Health Organization (18). This pooled serum contained 2470 mg of IgG per deciliter.

The antiserum, samples, and pooled human serum containing IgG were diluted with polyethylene glycol in phosphate-buffered physiological saline and then filtered through 0.22 μm (av pore diameter) Millipore filters (Millipore Corp., Bedford, Mass. 01730). The pH value of the polymer solution was checked before being used to prepare working solutions of antigen and antibody.

**Procedure**

An Aminco “Rotofil” dilutor was used to load the transfer discs. Ten microliters of diluted serum (25-fold dilution), 200 μl of diluent, and 400 μl of antiserum (20-fold dilution) were used. A four-track tape cartridge was used to program the computer to perform the necessary mixing and reading functions. The program was designed so that the solutions were mixed for 1 s, at 2 s before the signal was first measured from each cuvette. Mixing is necessary to keep larger antigen–antibody complexes from settling out of solution, and is not deleterious to the precision of the measurement (17). All reactions were carried out for 115 s at a rotor speed of 600 rpm and a temperature of 30 ± 0.05 °C.

Kinetic data were collected by sampling the signal from each cuvette at 15 s and 115 s. Data were printed out by the computer in relative intensity units, which were proportional to transmittance, with full-scale range from 0 to 2.5 relative intensity units. A standard curve was constructed by plotting change in relative intensity units during a fixed time interval vs. antigen concentration. Because the standard curves are nonlinear, unknown sample concentrations are determined by linear interpolation between known standard values.

![Bar graph](https://academic.oup.com/clinchem/article-abstract/20/10/1320/5677230)

**Fig. 1. Standard curve for the measurement of human IgG**

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<th>Table 1. Within-Run Precision</th>
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<td>Concentration</td>
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**Results**

*Standard curve.* Antigen–antibody reactions follow pseudo-first-order kinetics in a saline medium (19–21). Although it is not known whether the IgG–anti-IgG reaction follows pseudo-first-order kinetics when carried out in solutions containing polyethylene glycol, fixed-time kinetic analysis was chosen in the present studies. The validity of this method of analysis is demonstrated by the similarity between standard curves generated by fixed-time kinetics and those obtained by use of equilibrium light-scattering methods. Figure 1 shows the standard curve for the IgG–anti-IgG reaction for concentrations of antigen ranging from 0 to 2470 mg/dl. Adequate changes in relative intensity over a fixed-time kinetic period of 100 s are observed for samples in the normal range.

*Precision.* Previous investigation has shown that the laser-modified analyzer possesses exceptional photometric stability when used to perform light-scattering measurements (17), and this is reflected in the within-run precision for 56 replicate measurements of pooled normal and abnormal sera for IgG (Table 1).

*Correlation.* The correlation of results obtained for 40 sera by the present kinetic procedure with those for a continuous-flow nephelometric method in which a saline reaction medium is used (22) is shown in Figure 2. The good correlation demonstrates the accuracy of the present method.
The kinetic nephelometric method for serum IgG has a number of advantages over other methodologies. Because the measurement is a kinetic one, neither reagent nor sample blanks are necessary for accurate results. The volume of antisem required for the kinetic determination is greater than that required for a continuous-flow method, but further refinement of the methodology and instrumentation should overcome this objection. The kinetic method is now about 20% more costly (reagent cost) than is the continuous-flow equilibrium method, but cost would be reduced by analysis time saved by the kinetic method: 14 samples can be measured in 115 s, even less time than required for conventional analysis in which a reaction enhancer is used. Collection and manipulation of data is considerably simpler and faster than in conventional methodology.

There are as yet no studies of potential interference from nonspecific aggregation of rheumatoid factor and (or) preformed antigen–antibody complexes in the presence of polyethylene glycol, or the binding of serum complement components to antigen–antibody complexes formed during the reaction. Even though these specific interferences have not been observed thus far, further investigations in this area are warranted.

Detection of antigen excess in the kinetic method is less straightforward than in the continuous-flow method. We have discussed (22) the possibility of using very early rates as a method for screening for antigen excess. This would require a reading to be taken as early as 3 s after initiation of the reaction, and a subsequent reading to be taken within 10 to 12 s after the first reading. Samples in large antigen excess exhibit a very rapid initial rate (0–5 s), which subsequently becomes constant, while a reaction in which the antigen concentration is low proceeds much slower initially (0–5 s) but continues to show increase in light scattering over a longer time period.

Preliminary experiments show positive reactions for concentrations of IgG as low as 100 ng/ml in the reaction mixture, and indicate the possibility of quantitating serum proteins that are present in serum at concentrations in microgram or nanogram amounts per milliliter.

This present procedure represents the first automated kinetic nephelometric method for the measurement of a serum protein. Excellent precision is obtained, with good correlations being observed with a reference procedure. The use of rapid and accurate techniques for measuring serum proteins opens the possibility of serum protein “profiles” that would be available on the same basis as enzyme profiles are now. Investigations are currently underway to improve existing instrumentation and to develop methods for other serum proteins.

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References


